APOPTOSIS: MORPHOLOGY AND MECHANISMS

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SUMMARY

Apoptosis is a distinct mode of cell death that is responsible for deletion of cells in normal tissues; it also occurs in specific pathological contexts. However, the terminology has become confusing, and apoptosis may mean different thing to different users of the term.

The discovery of this phenomenon led to enormous increase in papers on apoptosis, with the latest developments in this field concerning the genes involved in cell suicide and the using apoptosis as an approach to cancer therapy. The ultimate challenge is to assess the role of apoptosis in the pathogenesis of some human disease including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS.

Key words: *apoptosis, cell death, mechanisms, morphology*

ÖZET

Apoptosis, normal dokularda hücrelerin ortadan kaldırılmasından sorumlu bir hücre ölüm biçimidir. Ancak apoptosis terminolojisi karmaşık olduğundan, farklı kullanıcılar tarafından farklı yorumlar ortaya çıkmaktadır.

Bu fenomenin ortaya çıkması ile, apoptosis konusunda çok sayıda makaleler yayınlanmıştır. Hücre ölümünde rol oynayan genlerin ortaya konmasındaki gelişmeler ve apoptosisin kanser tedavisindeki önemine dair yaklaşımlar bu konunun güncelliğini arttırmıştır. Bu konuda başlıca hedefler, apoptosisin kanser, viral infeksiyonlar otoimmun hastalıklar, nörodejeneratif hastalıklar ve AIDS'teki patogenetik rolünün ortaya konmasıdır.

Anahtar sözcükler: Apoptosis, hücre ölumu, mekanizma morfoloji

Spontaneous cell death as a physiological event was first discussed by Fleming in 1885. He gave the name chromatolysis to the apoptotic process. The original concept of chromatolysis did survive among the embryologists and the concept of cell suicide surfaced for the first time after the lysosomes were discovered in the late 1950s. Free radical pathology appeared in 1960s, and then came apoptosis (1).

The critical experiment was published in 1972 by Kerr (2). He noticed a discrete drop-off of the cells after inducing liver atrophy that he called first shrinkage necrosis and a year later apoptosis. Wyllie et al in 1984 linked the ladder pattern of the nuclear DNA with the phenomenon of apoptosis and thereby added a specific biochemical marker to the distinctive morphological changes of apoptotic cells(1,2, 3).

Necrosis is a general term referring to morphologic stigmata seen after a cell has passed the point of no return. The point at which the cells are irreversibly injured is difficult to define; instead we define cell death by later events, morphological changes called necrosis (1, 4, 5). Necrosis is signalled by irreversible changes in the nucleus (karyolysis, pyknosis, and karyorhexis) and in the cytoplasm (condensation and intense eosinophilia, loss of structure, and fragmentation). These are the features of the necrosis whatever the mechanism of the cell death, which may be ischemia, toxins, heat, mechanical trauma, or even apoptosis. Used in this way, apoptosis is one form of necrosis (apoptotic necrosis) (1).

Although the terminology is not perfect, the two best known modalities of cell demise are cell death by suicide (apoptosis) and cell death by murder (accidental cell death). The latter can be named as ischemic cell death and its necrosis as ischemic (coagulative) necrosis. However the rapidly developing tale of apoptosis warns us that generalizations are dangerous because, first, cell suicide does not always take the form of the apoptosis; second cell murder by cytotoxic lymphocytes and accidental causes, such as mild heat and toxic agents, leads to apoptosis; third there seem to several varieties of apoptosis and forth different cell types may follow different rules (1, 4, 5, 6,).

MORPHOLOGY

Apoptosis characteristically affects scattered single cells, not groups of adjoining cells. The chromatin becomes pyknotic and packed into smooth masses applied against the nuclear membrane (marginalization of the chromatin), creating curved profiles (1, 3, 7). The nucleus may also break up (karyorhexis) and the cell emits processes (the budding phenomenon). These processes tend to break off and become apoptotic bodies, which may be phagocytized by macrophages or neighbouring cells such as epithelial cells or remain free. The apoptotic cells do not induce an inflammatory response, even when they are present in large numbers. The rapid phagocytosis of apoptotic bodies is of critical importance in preventing inflammation (6).

Ultrastructurally, the endoplasmic reticulum dilates and a series of crater-like cavities appear where the dilated cysternae fuse with the cell surface. In contrast to the cell death by other means, there is little or no swelling of mitochondria or other organelles (3, 7). Electron microscopy is rarely applied for quantitation of apoptosis because of being an expensive and time consuming procedure.

On light microscopic examination with routine hematoxylene and eosin-stained sections, apoptotic bodies can be identified (Figure 1). However, there are descrepancies about the reliability of the identification, such as there may be difficulties in identifying early apoptotic cells and occasionally apoptotic cells are lost from epithelial surfaces into lumina, where they eventually swell and rupture (3). The apoptotic index is determined by counting either 1000 cells or cells in 10-15 high power fields (8).

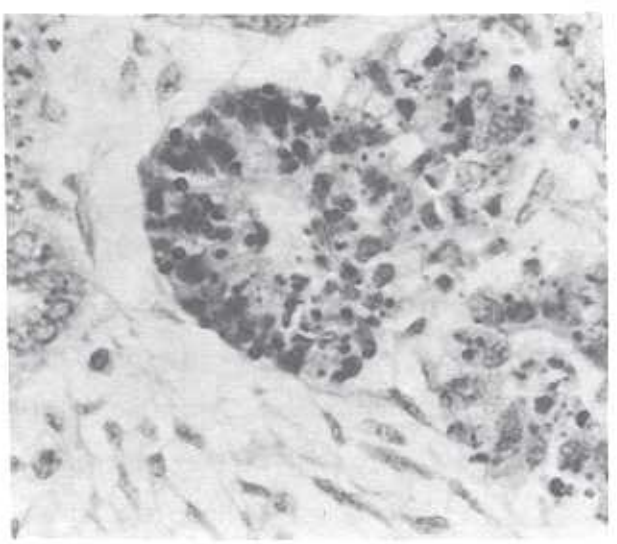


Figure 1. Light microscopic apoptotic morphology. Apoptotic renal tubule cells and many apoptotic bodies without any inflammatory response. H&E staining (x400 magnification).

Although a universal biochemical or antigenic marker for apoptosis is still lacking, fortunately a recent technical advance makes the identification of apoptosis at the single cell level, a matter of simple histochemistry, a method that takes the advantage of the DNA breaking points (nicks) exposing molecular endings that are chemically specific. Recently, a Tdt-mediated nick end-labeling technique (TUNEL) was described and have been proven to be helpful in the in situ detection of apoptotic cells (9). However, some mitotic cells may also be stained in this method while cells with obvious apoptotic morphology may not be stained and microwave treatment is effective in increasing the sensitivity (10). The characteristic strand breaks can also be detected by a modification of the nick translation procedure, which allows in situ end labeling (ISEL) of the DNA fragments (11). This method relies on the use of a DNA polymerase that acts on the naturally occuring strand breaks seen during apoptosis, allowing the incorporation of a non-isotopically labelled nucleotide. Also, flow cytometry may be performed for determining the apoptotic content. But this again results in contamination with undesired stromal cells (8).

EFFECTOR MECHANISMS

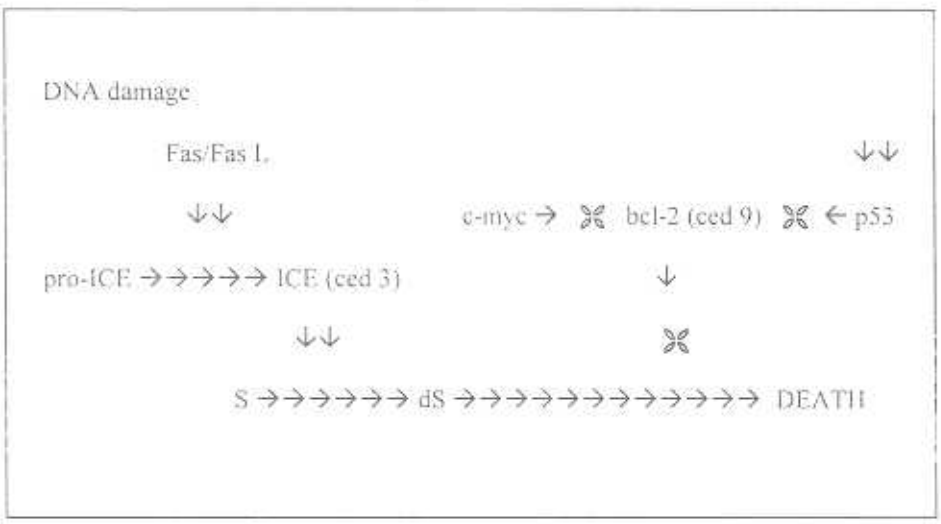
Many of the current hypotheses concerning effectors for apoptotic cell death (calcium dysregulation, oxidative stress, protease activation) are established mechanisms in necrosis. The occurrence of the similar features in both morphologies of cell death suggests that there is some degree of overlap in the triggers and perhaps initial cellular responses to injury, but segregation of these responses by timing, location, and in some cases strength of stimulus argues that both modes of cell death are significantly divergent responses.

Biochemically, the DNA is broken down into oligonucleosomal fragments that are multiplies of approximately 185 bp and gives the characteristic "ladder" on agarose gel electrophoresis. Selective activation of calcium-magnesium sensitive endogenous endonucleases appear to be responsible not only for wide-spread chromatine cleavage but also for the major nuclear morphological changes (3, 12, 13, 14). Shortly before the onset of the chromatine changes, free cytosolic calcium rises to moderate levels of around 800nM. It is unknown whether the raised calcium level results from receptor-mediated process, or accompanies minor injury, and whether the source of calcium is external or internal (3).

The characteristic nuclear morphology in apoptosis can also be explained in the terms of cleavage of transcriptionally active ribozomal genes, with conservation of the nucleolin-rich fibrillar centre (12). The dependance of apoptosis on protein and RNA synthesis is compatible with the view that new transcription is involved in morphological changes including the shrinkage and condensation of the apoptotic cells. Some of the shrinkage and distortion of the contour of the apoptotic cells are attributable to activation of transglutaminase expressed by

apoptotic cells (3,7). The swift phagocytosis is the result of recognition by the phagocytic cell of new molecular structures revealed on the surface of the apoptotic cell (1,7).

The apoptotic process is under genetic control and can be triggered by an internal clock, or by a variety of extracellular agents. There are several examples of specific genetically determined apoptotic pathways. The most compelling example of such a cell death pathway comes from studies of the nematode Caenorhabditis elegans (Figure 2). The C.elegans gene ced-9 protects against apoptosis and appears to be homologous to the proto-oncogene bcl-2 which encodes a protein localized to the outer mitochondrial, nuclear, endoplasmic reticulum membranes. It inhibits ced-3, a homologous gene to a mammalian gene, the interleukin converting enzyme (ICE). The apoptotic processes including cell shrinkage, surface changes permitting phagocytosis and endonuclease activation are initiated through the CED 3 / ICE-like proteases. However we lack the genetic evidence proving that high levels of ICE expression and ICE-like proteases induce apoptosis. Moreover, there is an alternative hypothesis that bcl-2 protects cell from apoptosis by inhibiting lipid peroxidation even in the presence of reactive oxygen intermediates (15, 16, 17). Bcl-x and mcl-1 are the other members of the bcl-2 family. There are also viral homologues, and bcl-2 interacts with other proteins such as R-ras, having several alternative partners (18).



( ) identifies homologues
↓↓ activation
→ ⌘ inhibition
S: substrate
dS: proteolytic product

Figure 2. Specific defined pathways controlling apoptosis elucidated from genetic studies in C.elegans as well as spontaneous mutations in higher organisms.

The functional integrity of bcl-2 depends on its ability to heterodimerize with bax, a bcl-2 binding protein. Mutations involving two critical amino acids (Gly 145 and Trp188) at bcl-2 destroys this ability. The mutation in codon 145 is a single Gly-to-Glu substitution. On the other hand, the bax homodimers acts in opposition to the apoptotic suppressive effect of bcl-2. The ratio of bax/bcl-2 could be therefore critical in determining the fate of the cell in response to the stimuli that can induce apoptosis (15,18,19,20).

Bcl-2 does not inhibit apoptosis in all circumstances, it fails to block apoptosis induced by cytotoxic T-lymphocytes (17). Nevertheless, stimuli such as fas-ligand receptor interactions and the p53 tumor suppressor protein-dependant pathway induced by genotoxic agents and radiation can modulate apoptosis (Figure 2). The fas-apo 1 gene, a cell surface molecule belonging to the tumor necrosis factor-receptor family initiates a sequence of events that is probably finally mediated by ICE-like proteases. In the immune system, Fas and fas ligand are involved in down-regulation of immune reactions as well as in T-cell mediated cytotoxicity. Frequently up-regulation of p53 mediates a cell cycle arrest in G1. If DNA damage is extensive, p53 mediates apoptosis of that cell, preventing propagation of the damaged DNA. To what extent p53 is involved in regulating apoptosis is unknown, however it affects the levels of bcl-2 and bax (1, 3, 21).

New transcription of some genes that may be triggers for apoptosis has been sought for by a candidate gene strategy (3, 15). Expression of c-myc is of particular interest. On withdrawal of serum growth factors, up-regulation of c-myc induces apoptosis. The possible involvement of c-ras and the mutated ras oncogene expression in the initiation of apoptosis have been also critically reviewed.

CONCLUSION

In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death. Although much is known about the control of cell proliferation, less is known about the control of cell death.

Apoptosis is a morphologically distinct form of cell death that occurs through a cell-intrinsic program regulated by many different signals that originate from genetic and biochemical pathways.

Recent evidence suggests that alteration in cell survival contribute to the pathogenesis of a number of human diseases, including cancer, viral infections, autoimmune diseases, neurodegenerative disorders, and AIDS. Treatments designed to specifically alter the apoptotic treshold may have the potential to change the natural progression of some of these diseases.

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